analog of a natural substrate of the NS3 protease was inhibitory led us to the peptide analogs of the present invention.

At page 106, lines 1 through 11; replace the paragraph with the following:

The substrate used for the HCV NS3 protease radiometric assay, DDIVPC-SMSYTW [SEQ. ID NO. 2], is cleaved between the cysteine and the serine residues by the enzyme. The sequence DDIVPC-SMSYTW [SEQ. ID NO. 2] corresponds to the NS5A/NS5B natural cleavage site in which the cysteine residue in P2 has been substituted for a proline. The peptide substrate DDIVPC-SMSYTW [SEQ. ID NO. 2] and the tracer biotin-DDIVPC-SMS[^{125}I-Y]TW [SEQ. ID NO. 3] were incubated with the recombinant NS3 protease in the absence or in the presence of inhibitors. The separation of substrate from products was performed by adding avidin-coated agarose beads to the assay mixture followed by filtration. The amount of SMS[^{125}I-Y]TW [SEQ. ID NO. 4] product found in the filtrate (with or without inhibitor) allowed for the calculation of the percentage of substrate conversion and of the percentage of inhibition.

At page 106, lines 19 through 25; replace the paragraph with the following:

Substrate: DDIVPC-SMSYTW [SEQ. ID NO. 2], 25 μM final concentration (from a 2 mM stock solution in DMSO stored at -20°C to avoid oxidation).
Tracer: reduced mono-iodinated substrate(biotin-DDIVPC-SMS[^{125}I-Y]TW) [SEQ. ID NO. 3] (≈ 1 nM final concentration).
HCV NS3 protease type 1b, 25 nM final concentration (from a stock solution in 50 mM sodium phosphate, pH 7.5, 10% glycerol, 300 mM NaCl, 5 mM DTT, 0.01% NP-40).

At page 107, lines 18 through 32; replace the paragraph with the following:

The enzyme was cloned, expressed and prepared according to the protocol described in Example 37. The enzyme was stored at -80°C, thawed on ice and diluted just prior to use in the assay buffer containing the NS4A cofactor peptide.
The substrate used for the NS3 protease/ NS4A cofactor peptide radiometric assay, DDIVPC-SMSYTW [SEQ. ID NO. 2], is cleaved between the cysteine and the serine residues by the enzyme. The sequence DDIVPC-SMSYTW [SEQ. ID NO. 2] corresponds to the NS5A/NS5B natural cleavage site in which the cysteine residue in P2 has been substituted for a proline. The peptide substrate DDIVPC-SMSYTW [SEQ. ID NO. 2] and the tracer biotin-DDIVPC-SMS[^{125}I-Y]TW [SEQ. ID NO. 3] are incubated with the recombinant NS3 protease and the NS4A peptide cofactor KKGSVVIVGRIILSGRK [SEQ. ID NO. 5] (molar ratio enzyme: cofactor 1:100) in the absence or presence of inhibitors. The separation of substrate from products is performed by adding avidin-coated agarose beads to the assay mixture followed by filtration. The amount of SMS[^{125}I-Y]TW [SEQ. ID NO. 4] product found in the filtrate allows for the calculation of the percentage of substrate conversion and of the percentage of inhibition.

At page 108, lines 4 through 14; replace the paragraph with the following:

Assay buffer: 50 mM Tris HCl, pH 7.5, 30% (*w/v*) glycerol, 1 mg/mL BSA, 1 mM TCEP (TCEP added just prior to use from a 1 M stock solution in water).
Substrate: DDIVPCSMSYTW [SEQ. ID NO. 2], 25 μM final concentration (from a 2 mM stock solution in DMSO stored at -20°C to avoid oxidation).
Tracer: reduced mono iodinated substrate biotin DDIVPC SMS[^{125}I Y]TW [SEQ. ID NO. 3] (~1 nM final concentration).
HCV NS3 protease type 1b, 25 nM final concentration (from a stock solution in 50 mM sodium phosphate, pH 7.5, 10% glycerol, 300 mM NaCl, 5 mM DTT, 0.01% NP-40).
NS4A Cofactor peptide: KKGSVVIVGRIILSGRK [SEQ. ID NO. 5], 2.5 μM final concentration (from a 2 mM stock solution in DMSO stored at -20°C).

At page 109, line 10 through page 110, line 8; replace the paragraph with the following:

The NS2-NS5B-3' non coding region was cloned by RT-PCR into the pCR®3 vector (Invitrogen) using RNA extracted from the serum of an HCV genotype 1b infected individual (provided by Dr. Bernard Willems, Hôpital St-Luc, Montréal, Québec, Canada). The NS3-NS4A DNA region was then subcloned by PCR into the pFastBac™ HTa baculovirus expression vector (Gibco/BRL). The vector sequence includes a region encoding a 28-residue N-terminal sequence which contains a hexahistidine tag. The Bac-to-Bac™ baculovirus expression system (Gibco/BRL) was used to produce the recombinant baculovirus. The full length mature NS3 and NS4A heterodimer protein (His-NS3-NS4AFL) was expressed by infecting 10^6 Sf21 cells/mL with the recombinant baculovirus at a multiplicity of infection of 0.1-0.2 at 27°C. The infected culture was harvested 48 to 64 h later by centrifugation at 4°C. The cell pellet was homogenized in 50mM $NaPO_4$, pH 7.5, 40% glycerol (w/v), 2mM β-mercaptoethanol, in presence of a cocktail of protease inhibitors. His-NS3-NS4AFL was then extracted from the cell lysate with 1.5% NP-40, 0.5% Triton X-100, 0.5M NaCl, and a DNase treatment. After ultracentrifugation, the soluble extract was diluted 4-fold and bound on a Pharmacia Hi-Trap Ni-chelating column. The His-NS3-NS4AFL was eluted in a >90% pure form (as judged by SDS-PAGE), using a 50 to 400 mM imidazole gradient. The His-NS3-NS4AFL was stored at -80° C in 50 mM sodium phosphate, pH 7.5, 10% (w/v) glycerol, 0.5 M NaCl, 0.25 M imidazole, 0.1% NP-40. It was thawed on ice and diluted just prior to use.
The protease activity of His-NS3-NS4AFL was assayed in 50 mM Tris-HCl, pH 8.0, 0.25 M sodium citrate, 0.01% (w/v) n-dodecyl-β-D-maltoside, 1 mM TCEP. Five (5) μM of the internally quenched substrate anthranilyl-DDIVPAbu[C(O)-O]-AMY(3-NO_2)TW-OH [SEQ. ID NO. 6] in presence of various concentrations of inhibitor were incubated with 1.5 nM of His-NS3-NS4AFL for 45 min at 23°C. The final DMSO concentration did not exceed 5.25%. The reaction was terminated with the addition of 1M MES, pH 5.8. Fluorescence of the N-terminal product was

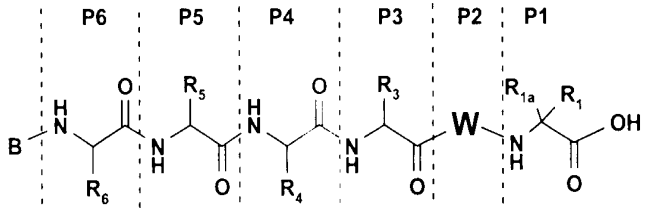
monitored on a Perkin-Elmer LS-50B fluorometer equipped with a 96-well plate reader (excitation wavelength: 325 nm; emission wavelength: 423 nm). A non-linear curve fit using the Hill model was then applied to the % inhibition-concentration data and 50% effective concentration (IC_{50}) was calculated through the use of SAS (Statistical Software System, SAS Institute Inc., Cary, N.C.).

At page 111, lines 12 through 29; replace the paragraph with the following:

The specificity of the compounds was determined against a variety of serine proteases: human leukocyte elastase, porcine pancreatic elastase and bovine pancreatic α-chymotrypsin and one cysteine protease: human liver cathepsin B. In all cases a 96-well plate format protocol using a colorimetric p-nitroaniline (pNA) substrate specific for each enzyme was used. Each assay included a 1 h enzyme-inhibitor pre-incubation at 30°C followed by addition of substrate and hydrolysis to ≈30% conversion as measured on a UV Thermomax® microplate reader. Substrate concentrations were kept as low as possible compared to K_M to reduce substrate competition. Compound concentrations varied from 300 to 0.06 μM depending on their potency. The final conditions for each assay were as follows:
50mM Tris-HCl pH 8, 0.5 M Na_2SO_4, 50 mM NaCl, 0.1 mM EDTA, 3% DMSO, 0.01% Tween-20 with;
[100 μM Succ-AAPF-pNA [SEQ. ID NO. 7] and 250 pM α-chymotrypsin], [133 μM Succ-AAA-pNA and 8 nM porcine elastase], [133 μM Succ-AAV-pNA and 8 nM leukocyte elastase]; or
[100 mM $NaHPO_4$ pH 6, 0.1 mM EDTA, 3% DMSO, 1mM TCEP, 0.01% Tween-20, 30 μM Z-FR-pNA and 5 nM cathepsin B (the stock enzyme was activated in buffer containing 20 mM TCEP before use)].

At pages 114 through 126, replace Tables 1 through 3 with the following amended Tables 1 to 3:

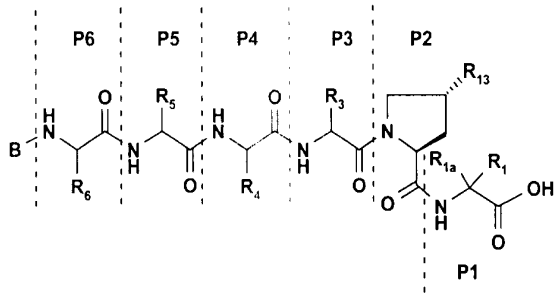
Table 1



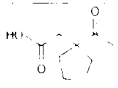
Tab. 1 Comp. #	B	P6	P5	P4	P3	W	P1	IC_{50} (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH^+)	AAA (%)	SEQ ID NO.
101	Ac	Asp	Asp	Ile	Val	Pro	Cys	46				703	113	8
102	Ac	Glu	Asp	Ile	Val	Pro	Cys	59				717	85.4 ± 1.6	9
103	DAD	---	Asp	Ile	Val	Pro	Cys	26				646	100.3 ± 1.8	10
104	Ac	Asp	D-Asp	Ile	Val	Pro	Cys	8.5				703	113.85 ± 4.9	-
105	Ac	Asp	D-Glu	Ile	Val	Pro	Cys	1.5				717	95.8 ± 0.8	-
106	Ac	Asp	Glu	Ile	Val	Pro	Cys	16*				717	98.8 ± 2.6	11
107	Ac	Asp	Val	Ile	Val	Pro	Cys	85*				687	85.9 ± 1.1	12
108	Ac	Asp	Tbg	Ile	Val	Pro	Cys	31				701	101.15 ± 1.65	13
109	Ac	Asp	Asp	Val	Val	Pro	Cys	80*				689	99.2 ± 5	14
110	Ac	Asp	Asp	Chg	Val	Pro	Cys	24*				729	102.95 ± 3.65	15
111	Ac	Asp	Asp	Tbg	Val	Pro	Cys	79				703		16
112	Ac	Asp	Asp	Leu	Val	Pro	Cys	92*				703	109.7 ± 6.9	17
113	Ac	Asp	Asp	Ile	Ile	Pro	Cys	56*				717	72.4 ± 2.4	18
114	Ac	Asp	Asp	Ile	Chg	Pro	Cys	50*				743	103.65 ± 3.8	19
115	Ac	Asp	Asp	Ile	Val	Abu	Cys	58*				691	59.4 ± 2.85	20
116	Ac	Asp	Asp	Ile	Val	Leu	Cys	16*				719	95.4 ± 1.5	21

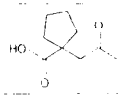
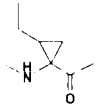
Tab. 1 Comp. #	B	P6	P5	P4	P3	W	P1	IC_{50} (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH^+)	AAA (%)	SEQ ID NO.
117	Ac	Asp	Asp	Ile	Val	Phe	Cys	25*				753	99.6	22
118	Ac	Asp	Asp	Ile	Val	Val	Cys	133*				705	96.8 ± 1	23
119	Ac	Asp	Asp	Ile	Val	Ile	Cys	90				719	87.0 ± 3.0	24
120	Ac	Asp	Asp	Ile	Val	Ala	Cys	76*				677	N.S.	25
121	Ac	Asp	Asp	Ile	Val	Hyp(4-Bn)	Cys	1.7				809	101	26
122	Ac	Asp	Asp	Ile	Val	Pro	Abu	315				685	91.0 ± 4.5	27
123	Ac	Asp	Asp	Ile	Val	Pro	Nva	220	>300	>300		699	107.6	28
124	Ac	Asp	Asp	Ile	Val	Pro	AlGly	210				697	106.3 ± 8.2	29
125	Ac	Asp	Asp	Ile	Val	Pro	Acpe	210				711	94.02 ± 3.19	30
126	Ac	Asp	Asp	Ile	Val	Pro	Acca	45				683	100.2	31
127	Ac	Asp	Asp	Ile	Val	Pip	Nva	605*				713	107	32
128	Ac	Asp	D-Glu	Ile	Val	Pro	Nva	7.4				713	100.9 ± 3.6	-
129	Ac	Asp	Tbg	Ile	Val	Pro	Nva	270*				697	99.8 ± 0.6	33
130	DAD	---	Asp	Ile	Val	Pro	Nva	123				642	107	34
131	Ac	Asp	Glu	Chg	Glu	Glu	Cys	24						35
132	Ac	Asp	D-Glu	Chg	Glu	Glu	Acca	36						-
133	Ac	Asp	Glu	Chg	Val	Glu(OBn)	Acca	39						36

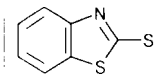
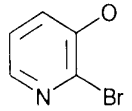
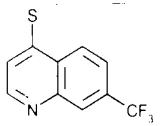
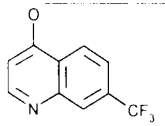
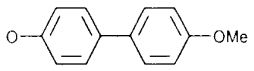
Table 2

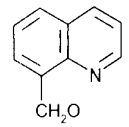
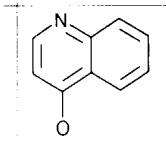
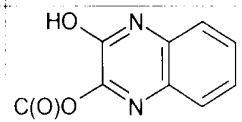
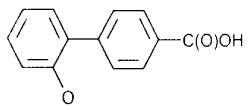
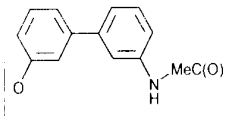
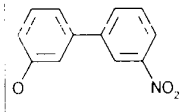


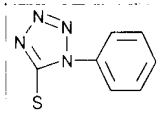
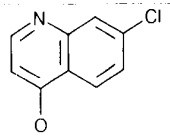
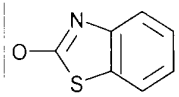
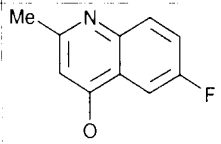
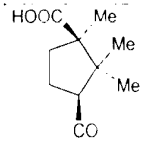
Tab.2 Comp.	B	P6	P5	P4	P3	R_{13}	P1	IC_{50} (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH^+)	AAA (%)	SEQ ID NO.
201	Ac	Asp	Asp	Ile	Val	O-Bn	Nva	7.2				805	107	37
202	Ac	Asp	D-Val	Ile	Val	O-Bn	Nva	0.93				789	103	-
203	Ac	Asp	D-Glu	Ile	Val	O-Bn	Nva	0.6	>300	>300	>300**	819	96.3 ± 1.7	-
204	Ac	Asp	Asp	Ile	Val	o-tolyl-methoxy	Nva	9.4*				819	95	38
205	Ac	Asp	Asp	Ile	Val	m-tolyl-methoxy	Nva	6.7*				819	98.7	39
206	Ac	Asp	Asp	Ile	Val	p-tolyl-methoxy	Nva	6.4*				819	101.9	40
207	Ac	Asp	Asp	Ile	Val	1-$NpCH_2O$	Nva	0.39				855	112	41
208	Ac	Asp	Asp	Ile	Val	2-$NpCH_2O$	Nva	0.71				855	104	42
209	Ac	Asp	Asp	Ile	Val	4-tert-butyl-phenyl)-methoxy	Nva	2.6				861	114	43
210	Ac	Asp	D-Glu	Chg	Val	O-Bn	Cys	0.033	>300	>300	>300	849	101.7 ± 5.4	-
211	Ac	Asp	D-Glu	Chg	Val	O-Bn	Nva	0.12				845	93.4 ± 2	-

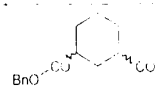
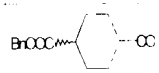
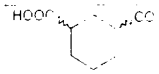
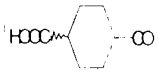
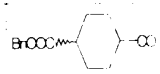
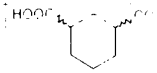
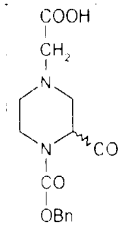
Tab.2 Comp	B	P6	P5	P4	P3	R_{13}	P1	IC_{50} (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH^+)	AAA (%)	SEQ ID NO.
212	Ac	Asp	D-Glu	Ile	Val	O-Bn	Acca	0.21	>300	>300		803	99.4 ± 2	-
213	Ac	Asp	D-Glu	Ile	Val	2-NpCH_2O	Nva	0.036				869	101.8	-
214	Ac	Asp	D-Glu	Chg	Val	2-NpCH_2O	Nva	0.028	>300	>300	>300 >300**	895	104.1	-
215	Ac	Asp	D-Glu	Chg	Val	1-NpCH_2O	Acca	0.014				879	---	-
216	Ac	Asp	Asp	Ile	Val	Bn	Nva	60				789	100.6 ± 0.8	44
217	Ac	Asp	Asp	Ile	Val	$Ph(CH_2)_3$	Nva	3				818	94.6 ± 3	45
218	Ac	Asp	D-Glu	Ile	Val	O-Bn	Nva	0.49				910	111.2	-
219	Ac	---	Asp	Ile	Val	1-NpCH_2O	Nva	2.3				740	95.7	46
220	DAD	---	---	N(Me)Ile	Val	1-NpCH_2O	Nva	31				697	---	-
221	DAD	---	---	Ile	Val	1-NpCH_2O	Nva	22				683		-
222	DAE	---	---	Ile	Val	1-NpCH_2O	Nva	20				698	N.S.	-
223		---	---	Ile	Val	1-NpCH_2O	Nva	51				737	N.S.	-

Tab.2 Comp	B	P6	P5	P4	P3	R_{13}	P1	IC_{50} (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH^+)	AAA (%)	SEQ ID NO.
224		---	---	Ile	Val	1-NpCH_2O	Nva	56				737	N.S.	-
225	Ac	---	---	Ile	Val	1-NpCH_2O	Nva	45				929	---	-
226	DAE	---	---	Chg	Val	1-NpCH_2O	Acca	0.76				707	---	-
227	Ac	---	---	Chg	Val	1-NpCH_2O	Acca	3	>600			635		-
228	Ac	---	---	Chg	Val	O-Bn		35	>600			613.4		-
230	Ac	Asp	Asp	Ile	Val	Ph$(CH_2)_3$	Nva	3.3				818		47
231	Ac	---	---	Chg	Chg	1-NpCH_2O	Acca	2.6				675.4		-
232	$AcOCH_2$-C(O)	---	---	Chg	Chg	1-NpCH_2O	Acca	1.4						-
233	Ac	Asp	Glu	Ile	Val	(3I-Ph) CH_2O	Acca	0.14				929.2		48
234	Ac	---	---	Chg	Chg	O-Bn	Acca	41						-
235	Boc	---	---	Chg	Chg	1-NpCH_2O	Acca	12						-
236	Ac	---	Gly	thioxo-Ile	Val	1-NpCH_2O	Nva	4.0				720 (M+Na)		-
237	DAE	---	---	Ile	Val	1-NpCH_2O	Acca	5.5				598		-

Tab.2 Comp	B	P6	P5	P4	P3	R_{13}	P1	IC_{50} (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH^+) (M+Na)	AAA (%)	SEQ ID NO.
238	Ac	---	---	Chg	Val	(4Br-Ph)O	Acca	27	195					-
239	Ac	---	---	Chg	Val	(2Br-Ph)O	Acca	27						-
240	Ac	---	---	Chg	Val	(3Br-Ph)O	Acca	42						-
241	Ac	---	---	Chg	Val		Acca	18						-
242	Ac	---	---	Chg	Val	(4Br-Ph)S	Acca	36						-
243	Ac	---	---	Chg	Val		Acca	35						-
244	Ac	---	---	Chg	Val		Acca	10						-
245	Ac	---	---	Chg	Val		Acca	5.0						-
246	Ac	---	---	Chg	Val		Acca	33						-
247	Ac	Asp	Asp	Ile	Val	$Ph(CH_2)_2$	Nva	10				803.6	119±1	49

Tab.2 Comp	B	P6	P5	P4	P3	R_{13}	P1	IC_{50} (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH^+)	AAA (%)	SEQ ID NO.
248	Ac	---	---	Chg	Chg		Acca	3.6						-
249	Ac	---	---	Chg	Val	(4I-Ph)O	Acca	9.7						-
250	Ac	---	---	Chg	Val		Acca	4.5						-
251	Ac	---	---	Chg	Val		Acca	13						-
252	Ac	---	---	Chg	Val	1-NpCH$_2$O	Nva	20				651.4	91±1	-
253	Ac	---	---	Chg	Val		Acca	28						-
254	Ac	---	---	Chg	Val		Acca	5.1						-
255	Ac	---	---	Chg	Val		Acca	4.5						-

Tab.2 Comp	B	P6	P5	P4	P3	R_{13}	P1	IC_{50} (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH^+)	AAA (%)	SEQ ID NO.
256	Ac	---	---	Chg	Val		Acca	11						-
257	Ac	---	---	Chg	Val		Acca	2.2	>300					-
258	Ac	---	---	Chg	Val		Acca	16						-
259	Ac	---	---	Chg	Val		Acca	28						-
260	Ac	Asp	D-Glu	Ile	Val	O-Bn	Cys	0.18						-
261	Ac	---	---	Chg	Val	O-Bn	Cys	28						-
262	Ac	---	---	Ile	Val	1-NpCH_2O	Acca	40				631 (M+Na)		-
263		---	---	Ile	Val	1-NpCH_2O	Acca	17				771 (M+Na)		-

Tab.2 Comp	B	P6	P5	P4	P3	R_{13}	P1	IC_{50} (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH^+)	AAA (%)	SEQ ID NO.
264		---	---	Ile	Val	1-NpCH$_2$O	Acca	6.4				811		-
265		---	---	Ile	Val	1-NpCH$_2$O	Acca	10				811		-
266		---	---	Ile	Val	1-NpCH$_2$O	Acca	9.7				721.4		-
267		---	---	Ile	Val	1-NpCH$_2$O	Acca	12				721.4		-
268	Ac	---	---	Chg	Val	(3Br-Ph)CH$_2$O	Acca	24				665.1		-
269		---	---	Chg	Val	1-NpCH$_2$O	Acca	2.2				835.5 (M-H)		-
270		---	---	Chg	Val	1-NpCH$_2$O	Acca	2.0				745 (M-H)		-
271		---	---	Chg	Val	1-NpCH$_2$O	Acca	3.8						-
272	Ac	---	---	Chg	Val	(3,5-Br$_2$-Ph)CH$_2$O	Acca	27						-

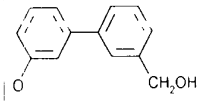
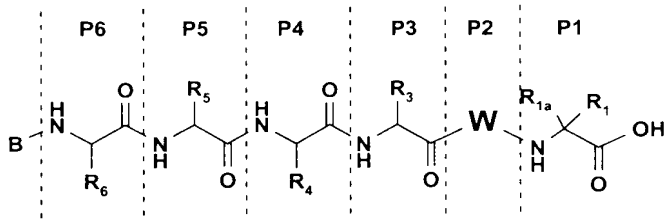
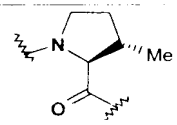
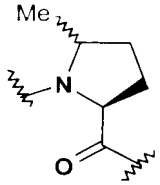
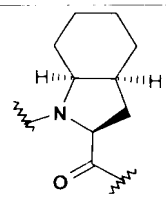
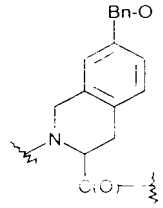
Tab.2 Comp	B	P6	P5	P4	P3	R_{13}	P1	IC_{50} (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH^+)	AAA (%)	SEQ ID NO.
273	Ac	Asp	Asp	Ile	Val	H	Nva	17.5						50
274	Ac	Asp	D-Val	Ile	Val	H	Cys	7.6						-
275	Ac	---	---	Chg	Val		Acca	6.2						-

Table 3



TAB 3 Cpd#	B	P6	P5	P4	P3	W	P1	IC_{50} (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH^+)	AAA (%)	SEQ ID NO.
301	Ac	Asp	Asp	Ile	Val		Nva	98*				713	99.8	51
302	Ac	Asp	Asp	Ile	Val		Nva	89*				713	102	52
303	Ac	Asp	Asp	Ile	Val		Nva	44*				753	104.4	53
304	Ac	---	---	Chg	Val		Acc a	1.1						-

Please insert the attached paper Sequence Listing after the Abstract on page 185.

IN THE CLAIMS:

Please amend the claims as follows: